PHOTOPERIODICALLY INDUCED TESTICULAR RECRUDESCENCE IN THE TRANSEQUATORIAL MIGRANT *DOLICHONYX* RELATIVE TO NATURAL PHOTOPERIODS [1]

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Some annual cycles of many birds of the northern hemisphere are known to be controlled by seasonal changes in the length of the daily period of light, that is, by the natural photoperiod. Best known is the annual cycle of the male gonad (reviews by Burger, 1949; Farner, 1959, 1961). Transequatorial migrants present a special problem, since they twice annually pass from the shortening days of autumn in one hemisphere to the lengthening days of spring in the other. They never experience the short days of late autumn and early winter; every year they experience two spring-summer seasons, but they confine their nesting to just one of these. Obviously, if the annual day-length cycle plays a part in regulating their annual breeding cycle, then the mechanism must differ in some conspicuous way from that operative in a temperate-zone resident or migrant. Early speculations as to the nature of this difference (*e.g.,* Farner, 1954) focused on the post-breeding photorefractory phase of the cycle and assumed that the migrants would still be refractory on arrival in the other hemisphere, where then lengthening days might prolong refractoriness until the late summer.

A photoperiodic effect on a transequatorial migrant was first demonstrated (Engels, 1959) in the bobolink, *Dolichonyx oryzivorus,* which breeds in North America in June and July and spends the months of November through March in South America below the equator. The testicular cycle in this species shows a photorefractory phase which in some respects is like that of temperate-zone migrants, while in others it differs in a manner that suggests it would be terminated during the post-breeding, southward transequatorial migration (Engels, 1961, 1962). There is a suggestion that, following termination of refractoriness, initiation of recrudescence is greatly delayed in comparison with temperate-zone migrants on moderately long photoperiods, but apparently begins abruptly, without delay, on quite long photoperiods, as in temperate-zone migrants (Engels, 1964). It is the purpose of the present paper further to examine these last two points, and to attempt a general correlation of the experimental results with the events occurring in nature.

Accordingly, this report is divided into two main sections, the first of which describes the experiments and their results, and makes some comparisons with a temperate-zone migrant; following this is an account of the distribution of bobo-

links in South America, the time of occurrence of testicular recrudescence, and the relationship of the experimental results to the timing of this event in terms of day-lengths encountered in nature.

Materials and Methods

Ninety-six male bobolinks were used in these experiments. Of these, sixty-two were caught in May during northward migration, hence all were about one year old or older. Thirty-four birds were caught in September during southward migration; many of these were adults but some were immature. All the autumn captures and nine of the spring captures were taken in North Carolina; fifty-three birds were caught in Florida in early May and transported to Chapel Hill by commercial airline. All the birds were held in an outdoor aviary until the start of artificial lighting in early October, and hence were exposed until then to the natural photoperiods of Latitude 36° N.

At the beginning of October the birds were confined in cages in ventilated, light-tight compartments in the attic of the laboratory. "White" fluorescent lamps, controlled by automatic time switches, provided from 95 to 135 foot candles of light on the floor of each cage, depending on its position in the compartment. It is presumed that these intensities are supramaximal for the response studied. Temperature was not controlled; in the unheated attic, within the lighted compartments, the temperature ranged from 18° to 25° C, approximately, during the course of the experiments. There was a day-night difference of about 2° C due to heat from the lamps. A food hopper and two water tubes provided ample food and water. The food consisted of a commercial, pelleted mash prepared for "game" birds.

During the first five weeks of artificial lighting, all birds were exposed to daily photoperiods of 12.5 hours (0545h–1815h). Previous study had shown that photo-refractoriness would be terminated under such a lighting regime (Engels, 1962). Four of the September captures and six of the May captures were then killed; the weights of their testes indicated the approximate weights of testes of the remaining birds at the start of exposure to longer photoperiods.

In all my earlier studies on bobolinks, a "long" day of 14 hours of light was used and the occurrence of recrudescence was scored for each bird when it became apparent that the horny epidermis of the beak was darkening. This black pigmentation, characteristic of the male in nuptial dress, is caused by the male sex hormone (Engels, 1959). We did not know, of course, what was happening previous to the appearance of pigmentation. In the present experiment the remaining thirty autumn-caught birds were exposed to 14-hour daily photoperiods and, beginning after six weeks, were sampled at 14-day intervals. Four of the spring-caught birds were killed after only sixteen days on 14-hour photoperiods. The remaining 52 spring-caught birds were divided into 4 groups which were exposed to longer photoperiods, namely 18 hours (six birds), 17 hours (twelve birds), 16 hours (seventeen birds) and 15 hours (seventeen birds). These were sampled first after 16 days, when all the "18-hour" birds had developed beak pigmentation; further sampling was done on a plan intended to uncover differences between photoperiods in the rate of recrudescence.

FIGURE 1. Recrudescence of bobolink testes, as indicated by weight, when exposed to 14-hour photoperiods beginning in early November. Sloping line indicates a *k* value of 0.04 beginning at day 80.

The birds were killed by decapitation, the testes removed and fixed in Helley's fluid; subsequently, after some time in 70% alcohol, they were weighed to the nearest 0.1 mg.

RESULTS

The results are presented in Figures 1 and 2. It will be convenient to consider them in three steps: first, the data from the 14-hour photoperiods, next the data from the 18-hour photoperiods and lastly the data from the photoperiods intermediate to these two.

1. *14-hour photoperiods (Figure 1)*

There is no apparent upward trend in the testicular weights until 84 days after the first 14-hour photoperiod, when one of the four birds shows that recrudescence has begun, with testes weighing 8.9 mg. After 114 days the testes of one of four birds are still inactive (only 1.2 mg), two may have just begun active growth (about 3 mg), while one has reached 80 mg. The final two specimens, killed after 130 days, were both undergoing testicular recrudescence, although the testes of one weighed only 11.1 mg. The results indicate that the initiation of recrudescence is delayed for several weeks, perhaps ten or more, after the first 14-hour photoperiod.

2. *18-hour photoperiods (Figure 2)*

The results are in sharp contrast to those of the 14-hour group. Recrudescence apparently began immediately; after only 16 days each of six birds tested had
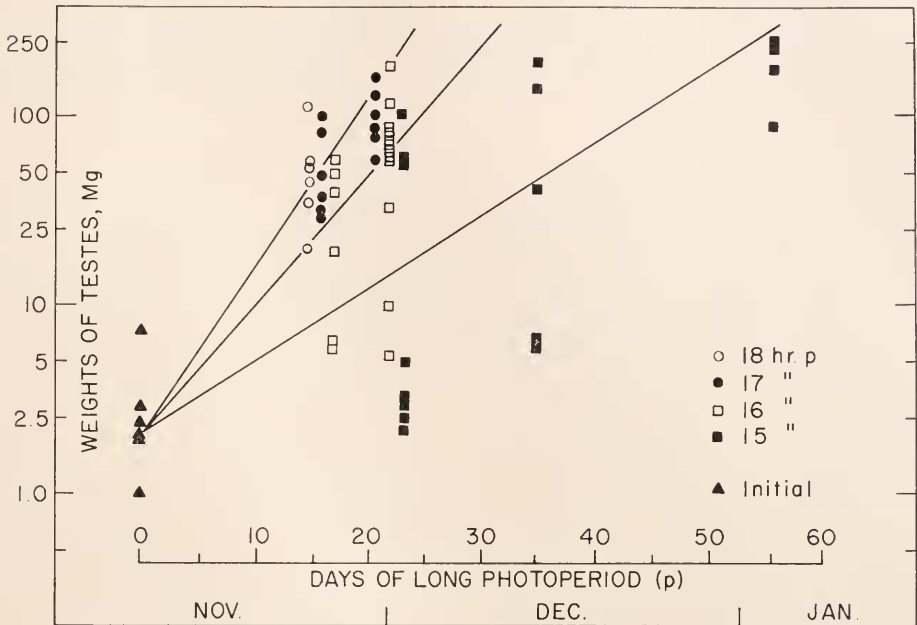
FIGURE 2. Recrudescence of bobolink testes, as indicated by weight, when exposed in early November to photoperiods of 15, 16, 17 and 18 hours. Lines indicate k values of 0.09, 0.07 and 0.04 (see text).

already developed the black beak pigmentation indicative of the production of male sex hormone, and testicular weights ranged from about 20 mg to more than 100 mg. Four birds of similar history, which were killed at the same time after 16 days on 14-hour photoperiods, each had testes weighing less than 2.5 mg (average 1.75 mg, Figure 1).

3. 17-hour, 16-hour and 15-hour photoperiods (Figure 2)

Although the "17-hour" birds lagged a little behind the "18-hour" birds with respect to average time of development of beak pigmentation, the samples do not show any marked difference in the rate of recrudescence. On the 16th day, weights of testes of six birds in the "17-hour" group ranged from about 30 mg to just over 100 mg (compared with 20 mg to 115 mg in the "18-hour" group). At this time there still were six birds in the "17-hour" group that did not show beak pigmentation; by the 22nd day their testicular weights considerably exceeded in value those of the first sample, ranging from 60 mg to 164 mg, with an increase in the mean from 56 mg to 104 mg.

The rate of recrudescence in the "16-hour" group was definitely lower than in the previous two groups, weights of six pairs of testes on the 16th day ranging from 6 mg to 60 mg. By the 22nd day some were still quite small (5.5 mg and 10 mg in two birds) but a number were of the same size as in "17-hour" birds at this time (17-hour: 60 mg to 164 mg; 16-hour: 59 mg to 189 mg). The first sample of the "15-hour" birds was taken on this day; the range of testicular

weights (2.3 mg to 105 mg) is somewhat lower than in the "16-hour" group, and the median is much lower (5 mg compared with 67 mg), both indicating a lower rate of recrudescence. After 35 days testes of two birds still were less than 10 mg, but testes of the other three birds weighed from 90 mg to just under 250 mg.

Initial weights (Figure 2)

The testes of five (spring-caught) birds had an average weight of 2.0 mg (1.0 mg to 2.8 mg) after the five weeks of 12.5-hour photoperiods in the early fall. In a sixth one the testes astonishingly were enlarged and weighed 7.2 mg. At this time I cannot account for this exceptional individual. Nevertheless the general conclusions presented above and the analysis to follow, which exclude any consideration of this one bird, seem to me to be truly valid.

Comparison with a temperate-zone migrant (Figures 1 and 2)

The sloping lines in Figures 1 and 2 are not fitted to the plotted data; rather, they are drawn to three selected values for average daily increment in the logarithm of weights in milligrams (k). These selected values approximate those determined for *Zonotrichia leucophrys,* a temperate-zone migrant, for photoperiods of 18 hours (0.09), 16 hours (0.07) and 14 hours (0.04) (Farner and Wilson, 1957). They are intended only to serve as visual guidelines, to aid in evaluating the presented bobolink data and in making general, qualitative comparisons between these two species.

The data both from 18-hour and from 17-hour photoperiods indicate an immediate, linear response of the same general order of magnitude as in *Z. leucophrys* for these photoperiods. The same probably may be said of the data for 16-hour photoperiods. At least some of the birds on 15-hour photoperiods also indicate an immediate response, with recrudescence proceeding then at a somewhat low rate. On 14-hour photoperiods, however, quite unlike temperate-zone migrants, the testicular response definitely is delayed for several weeks, ten or more; but once recrudesecence is initiated, it proceeds at a rate which may perhaps be comparable to the rate of the immediate response of *Z. leucophrys* to this photoperiod (Fig. 1). The delay is rather to be expected in a transequatorial migrant; after all, there is an interval of about seven months (in this particular case) between arrival in the southern hemisphere in early November and the next courtship, mating and nesting in the following June in the northern hemisphere.

DISCUSSION

Annan (1963) not only still entertained the old view that this delay was the expression of a photorefractory condition, but cited results (Engels, 1962) with bobolinks as "supporting" the idea. The data now at hand for the longer photoperiods, which induce an immediate response, show that refractoriness had indeed been terminated by the preliminary treatment of five weeks of daily 12.5-hour photoperiods. Whatever the mechanism responsible for the delayed response to 14-hour photoperiods, it cannot be said to be photorefractoriness. Thus, the mechanism of recrudescence in this transequatorial migrant differs from that in a temperate-zone migrant *not* in a prolongation of the photorefractory period, but

first in the capacity to terminate refractoriness on much longer days (such as are encountered in the transequatorial passage), and secondly in the delayed response to moderately long photoperiods.

1. *Distribution from November through March in South America*

Meyer de Schauensee (1966, p. 441) states that the bobolink is a "winter resident in southern South America in Peru, eastern Bolivia, western Brazil (Amazonas; Mato Grosso; casually in Rio Grande do Sul), Paraguay and nothern Argentina to Tucumán, Córdoba, Santa Fe and Buenos Aires." The latitudinal limits thus lie in Peru and Argentina. In Peru bobolinks occur from Tujillo, Latitude 8° S., southward (Koepke, 1961). The Argentine limits as outlined above repeat the distributional data given by the Argentine ornithologist Olrog (1963). In reply to my inquiry, Olrog writes that "the normal southern limit goes over southern Tucumán, northern Córdoba, Santa Fe and Entre Rios." This would put them down about to Latitude 30°–32° S. He adds that "stragglers go farther south, eventually attracted by crops" but he believes that the "normal winter home is well in the subtropical lowlands and not in the temperate." The inclusion of the province of Buenos Aires in both lists (Meyer de Schauensee, 1966; Olrog, 1963) probably is based on a published record by Pereyra (1923); he lists a male collected in December, 1920, at Isla de Escobar, Province of Buenos Aires (about 34° S.?) and remarks that "en ciertas epocas" (Pereyra, 1923; p. 171) the species appears in this region in large flocks. In his letter to me Olrog says, "personally, I never saw a bobolink in the Buenos Aires province."

It appears, then, that during their sojourn in South America bobolinks occur at localities lying between Latitude 8° S. and Latitude 30° to 32° S., with occasional or sporadic wandering of some flocks farther southward, perhaps as far as to Latitude 35° S.

2. *Testicular recrudescence in nature in South America*

The evidence as to when testicular recrudescence occurs in nature is inferential and is based on museum specimens. From August to March male bobolinks are grossly indistinguishable from females. During the breeding season, however, the male is a strikingly colored bird of black, white and golden cream with a jet-black beak, totally unlike the streaked, pale brown and yellowish female with a pale, horn-colored beak. The molt to the nuptial plumage occurs in South America, beginning in late January or in February; it is completed sometime in March (exceptionally in late January), before the start of the prenuptial, northward migration. There is no evidence that the coloration of the new plumage is linked in any causal way with active testes. On the other hand, the nuptial beak pigmentation is so linked; it is caused directly by the male sex hormone (Engels, 1959). Hence, the appearance of this pigmentation is a sign that testicular recrudescence already is under way.

Of the November-March specimens in U. S. museums, none of the males has a darkened beak, although four are in full breeding plumage (31 January, 23 and 28 March and 1 April; all collected between 17° and 22° S.). There are twelve additional April males (all migrants) from South America, all in full breeding plumage. Of these, four have darkly pigmented beaks, eight do not. Those with
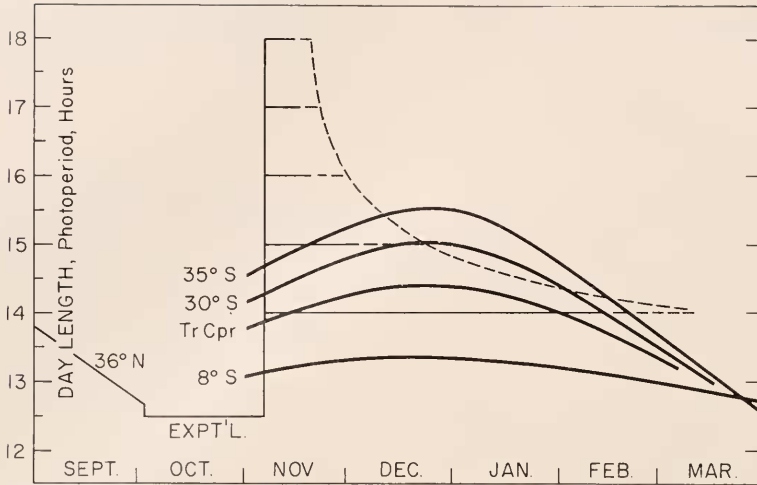
FIGURE 3.   Day-lengths (including civil twilight) experienced by bobolinks in South America (curved lines) and the photoperiods used in these experiments (straight lines). The broken-line curve approximates the occurrence of testicular regrowth to weights of about 50–100 mg, Tr Cpr—Tropic of Capricorn.

dark beaks were collected above the equator in Colombia, April 14–22, 1957; of those with pale beaks, five were taken on April 16, 1923, in Brazil about 4° below the equator, one on April 26, 1903 above the equator in Venezuela while one from Brazil and one from Venezuela lack precise dates.   Since, then, mid-April may approximate the first appearance of this pigmentation, the testes must remain inactive perhaps through March, with the initiation of recrudescence occurring at about the beginning of April.

3. *Day-lengths in South America and the photoperiodic response of male bobolinks* *(Figure 3)*

If the photoperiodic responses obtained in our experiments are to be meaningful, it is necessary to relate them to the natural photoperiods experienced by the species. Unfortunately, the results are not entirely unequivocal.

The most satisfactory "fit" of the experimental data to winter distribution has to do with the southern limits.   Since the winter range does not extend beyond Latitude 30° to 35° S., it is clear from Figure 3 that the males are never exposed, even at the December solstice, to days as long as or longer than 16 hours—the photoperiods which in our experiments induced almost immediate recrudescence. In effect, the response to these longer photoperiods would act selectively against a southward extension of the species' range into the higher latitudes characterized by these longer day-lengths.

On the other hand, the results of the experiment using 15-hour photoperiods would suggest that the winter range should not extend to Latitude 35° S.   Indeed, in an earlier experiment (Engels, 1962; Group E, p. 97), I did use photoperiods which simulated the daily change in day-length at Latitude 35° S.; testicular recrudescence was induced apparently by late December (beak pigmentation ob-

vious by mid-January), much too early compared with the natural timing. Fourteen-hour photoperiods approximate average natural day-lengths in the northerly part of the winter range, and they did greatly delay the onset of recrudescence, although not quite until the normal time.

In this connection, there is an interesting question (but one not yet investigated, as far as I know): whether, in the various species which show photoperiodic responses, there exist intraspecific differences in threshold or in rate of response between populations wintering at different latitudes. It is not unreasonable to think that such differences may exist. Of course we do not know where, in South America, our experimental birds had their winter homes. If they came from relatively low latitudes (north of the Tropic of Capricorn for example), and if there are differences in rates of recrudescence correlated with latitude, then the experimental results might approximate more closely those expected from a consideration of events in nature.

Summary

1. *Dolichonyx oryzivorus*, the bobolink, breeds in North America in June and July and spends the months of November through March in South America below the equator. Ninety-six males captured during migration were held in an outdoor aviary at Chapel Hill, North Carolina (Latitude 36° N) until start of artificial lighting.

2. Beginning in early October, they were exposed daily to 12.5-hour photoperiods for five weeks to terminate photorefractoriness. Thereafter, different groups were subjected to daily photoperiods of 14, 15, 16, 17 and 18 hours. These groups were sampled at intervals and progress of testicular recrudescence was determined by gonadal weights.

3. Evidence adduced from museum specimens indicates that testicular recrudesence begins about the end of March. In the experiments, recrudescence seemingly was initiated immediately, in November, on the longer photoperiods and proceeded at rates comparable to those shown by a temperate-zone migrant. Drastically different was the response to 14-hour photoperiods, initiation of recrudescence being delayed for ten or more weeks.

4. In South America bobolinks occur between Latitude 8° S and Latitude 30° to 35° S. Day-lengths at these latitudes never exceed 15.5 hours; so even at the December solstice males are not exposed to the longer photoperiods which brought about immediate recrudescence in the experiments. Because bobolinks do not come into breeding condition in the southern hemisphere, this response is viewed as a limiting factor in southward distribution. But since 15-hour photoperiods also induced early recrudescence, one would not expect the species to go as far south as Latitude 30° to 35°. This is the most puzzling lack of fit of the experimental results to nature.

5. Although 14-hour photoperiods greatly delay the onset of recrudescence, they do induce it earlier than its occurrence in nature, even though they are some-

what shorter than the average day-length occurring at the latitudes in the middle of the bobolink's winter range. Thus there is at best an imperfect fit of the experimental results to the normal timing.

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